PHYSIOLOGY OF INSECT DIAPAUSE. IV. THE BRAIN AND PROTHORACIC GLANDS AS AN ENDOCRINE SYSTEM IN THE CECROPIA SILKWORM

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In the previous papers of this series (Williams, 1946b, 1947, 1948a) an endocrine basis was described for the production and termination of pupal diapause in the Cecropia silkworm. The onset of diapause was correlated with a temporary failure of the brain in secreting a hormone required for the initiation of adult development. The ultimate release of this "brain hormone" was found to terminate dormancy and to set in motion the process of adult development. The brain was therefore viewed as the organ of primary control over the genesis and termination of diapause in the Cecropia silkworm.

After more detailed examination it became apparent that the brain hormone is fundamentally a tropic factor whose primary target within the diapausing pupa is a second endocrine tissue, the prothoracic glands. The latter, under stimulation of the brain hormone, then secrete the factor reacting with the pupal tissues to terminate diapause (Williams, 1947–1951). The brain and prothoracic glands were therefore considered to function as an endocrine system in controlling the pupal diapause. However, the role of the brain hormone was not fully clarified in the above-mentioned studies. There remained the possibility that the brain hormone might also act directly on the pupal tissues to condition their ultimate response to the prothoracic gland hormone.

The roles of the brain and prothoracic glands have therefore been examined in further detail in the present investigation. Attention has also been focussed on the possibility that, at earlier periods in the life history, the same hormonal system may control the moulting and pupation of the larval insect.

MATERIALS AND METHODS

The present report is based on a study of approximately two thousand Cecropia silkworms (*Platysamia cecropia*). In certain experiments other Lepidoptera, including *Telea polyphemus, Actias luna, Actias selene, Antheraea mylitta, Bombyx mori, Danaus plexippus, Lymantria dispar* and *Prodenia eridania*, were used as donors and recipients of various endocrine organs. The insects were reared in large numbers from eggs obtained from fertile females. In general, the management of the experimental animals was essentially the same as that described previously (Williams, 1946b).

Carbon dioxide anesthesia was used in all surgical procedures (Williams, 1946a). In experiments performed on pupae, surgical mortality was minimized by implanting several crystals of phenylthiourea and/or a few drops of cyanide Ringer's solution.[2] By temporarily blocking the enzyme tyrosinase, these agents served to protect postoperative pupae from toxic quinone intermediates which appear in the blood if the latter is permitted to darken as a result of the tyrosine-tyrosinase reaction. In certain experiments, a few crystals of streptomycin sulfate and potassium penicillin G were also implanted. These antibiotics virtually eliminated bacterial infection which otherwise complicates certain experiments, especially those performed on isolated pupal abdomens; their use was found to introduce no discernible complications.

The Prothoracic Glands as the Source of the Hormone Terminating Pupal Diapause

In order to induce the adult development of an isolated abdomen it is necessary to implant a brain from a previously chilled pupa, plus prothoracic glands obtained from either chilled or unchilled pupae (Williams, 1947). If the brain functions solely by activating the prothoracic glands, then it should be possible to accomplish this same end in the absence of the brain by implanting prothoracic glands which have already been activated by the brain hormone. Moreover, according to results reported previously (Williams, 1947), the glands should exist in this functional state when obtained from post-diapausing pupae during the first five days of adult development.

Table I

Endocrine activity of prothoracic glands removed at the outset of adult development and tested in brainless pupae or in pupal abdomens

Test preparation	Pairs of glands implanted into each	Number of experiments	Number developing
Pupal Cecropia abdomens	3 from Cecropia	2	1
	3 from Polyphemus	4	2
Brainless diapausing Cecropia pupae	2 from Cecropia	1	0
	2 from Polyphemus	2	0
	3 from Cecropia	2	1
	3 from Polyphemus	3	3
		14	7

Prothoracic glands were therefore removed from animals immediately after the onset of adult development and implanted into isolated pupal abdomens. Experiments of this type were complicated by the fragility and stickiness of the glands which, at the outset of adult development, begin to undergo rapid degeneration;

[2] Prepared daily by the addition of 1 part 0.1 M potassium cyanide solution to 9 parts of the insect Ringer's solution described by Ephrussi and Beadle (1936). At the pH of the insect, the cyanide exists almost wholly as HCN and within a few days is lost *via* the tracheal system.

ordinarily they could be isolated and implanted only as small fragments.   However, by using *Telea polyphemus* as donors, the difficulty was somewhat lessened, since, in this closely related diapausing species, the prothoracic glands are much easier to locate and isolate during the first few days af adult development.

A further serious complication was the high mortality among abdomens receiving such implants.   Notwithstanding the use of cyanide and phenylthiourea, the implanted glands caused a delayed darkening of the blood followed by the death
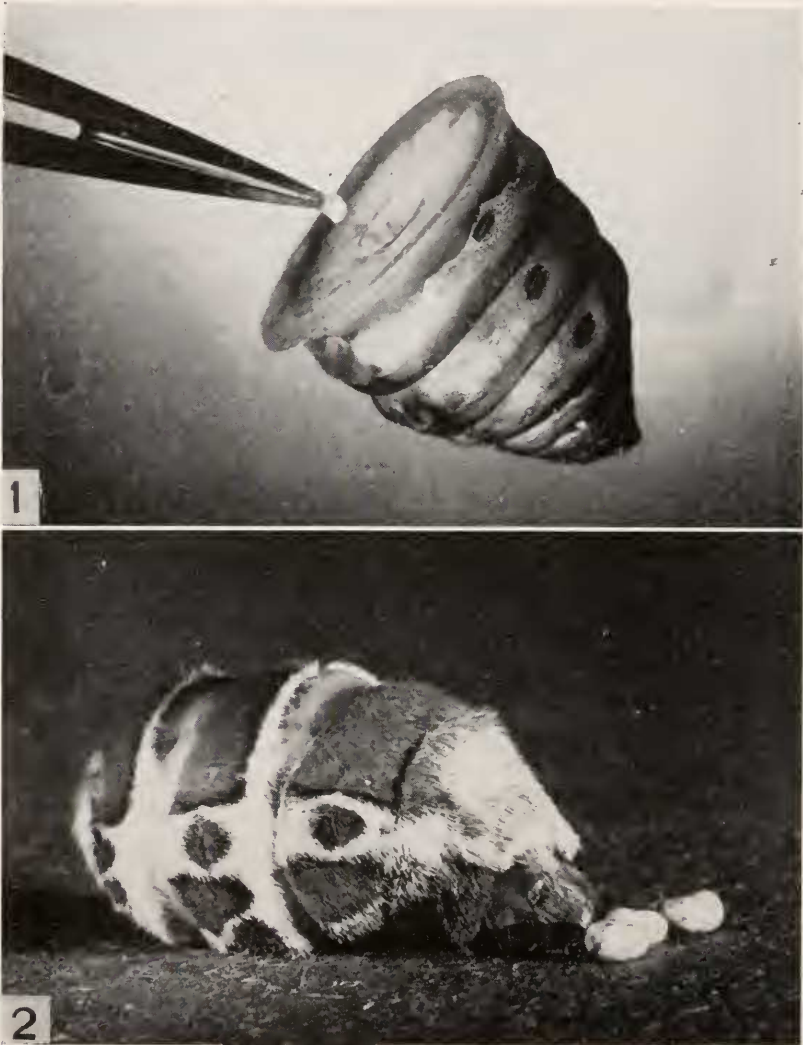

FIGURE 1.   Isolated abdomen of a diapausing Cecropia pupa sealed to a plastic slip. Prothoracic glands obtained from postdiapausing pupae are being implanted through a central hole in the slip.

FIGURE 2.   The preparation in Figure 1, after adult development.   The active prothoracic glands have evoked the metamorphosis of the abdomen; the latter is shown laying several eggs.

of the preparation. This result was not peculiar to the prothoracic glands, but was also commonly observed after implanting fragments of fat-body obtained from developing adults.

Of a total of 25 isolated pupal abdomens, only 6 survived longer than a week following the implantation of prothoracic glands obtained from developing animals (Fig. 1). However, as may be observed in Table I, three of these underwent prompt and complete adult development (Fig. 2).

A lower mortality was encountered in experiments testing the glands in brainless diapausing pupae rather than in isolated abdomens. Of a total of 8 viable preparations of this type, four underwent adult development (Table I).

In view of the injury and fragmentation of the glands during the experimental procedure, the high proportion of negative results is understandable. However, the fact that adult development occurred in 7 of the total of 14 viable preparations is, in itself, highly significant. It demonstrates that the pupal tissues do not require prior reaction with the brain hormone in order to respond to the prothoracic gland hormone.

Ligation Experiments on Mature Larvae

Efforts were made to determine whether the brain and prothoracic glands control pupation as well as adult development. In view of the difficulties inherent in surgical procedures on caterpillars, the technique of ligation was applied to mature fifth instar Cecropia silkworms.

As illustrated in Figure 3, two transverse ligatures were placed around each larva, one between the head and prothorax and the other between the second and third abdominal segments. In this manner each animal was subdivided into three blood-tight compartments—a cephalic compartment containing the brain, corpora cardiaca, and corpora allata; a primarily thoracic compartment containing the prothoracic glands; and an abdominal compartment containing no recognized endocrine glands.

The isolated head promptly died, but, as indicated in Table II, the behavior of the thoracic and abdominal compartments was determined by the stage of maturity of the silkworm at the time of ligation. Larvae subdivided just prior to the initiation of spinning underwent no further development, although both thoracic and abdominal compartments continued to live for about a month thereafter (Fig. 3). When the ligatures were applied during the first day of spinning, the abdomen remained larval, whereas the thorax either remained larval or pupated during the following two weeks. Silkworms subdivided during the second day of spinning retained larval abdomens, but all of the thoracic compartments underwent pupation about two weeks thereafter (Fig. 4).

The pupation of the larval abdomen became possible only when the ligatures were applied after the initiation of the prepupal stage. At 25° C. this stage begins four to five days after the initiation of spinning and is accompanied by the eversion of the wing disks and the retraction of the hypodermis from the larval cuticle. In the intact animal this retraction may be recognized by the withdrawal of the pigment granules underlying the transparent larval ocelli (Kühn and Piepho, 1936). Silkworms ligated at the first sign of pigment retraction retained larval abdomens
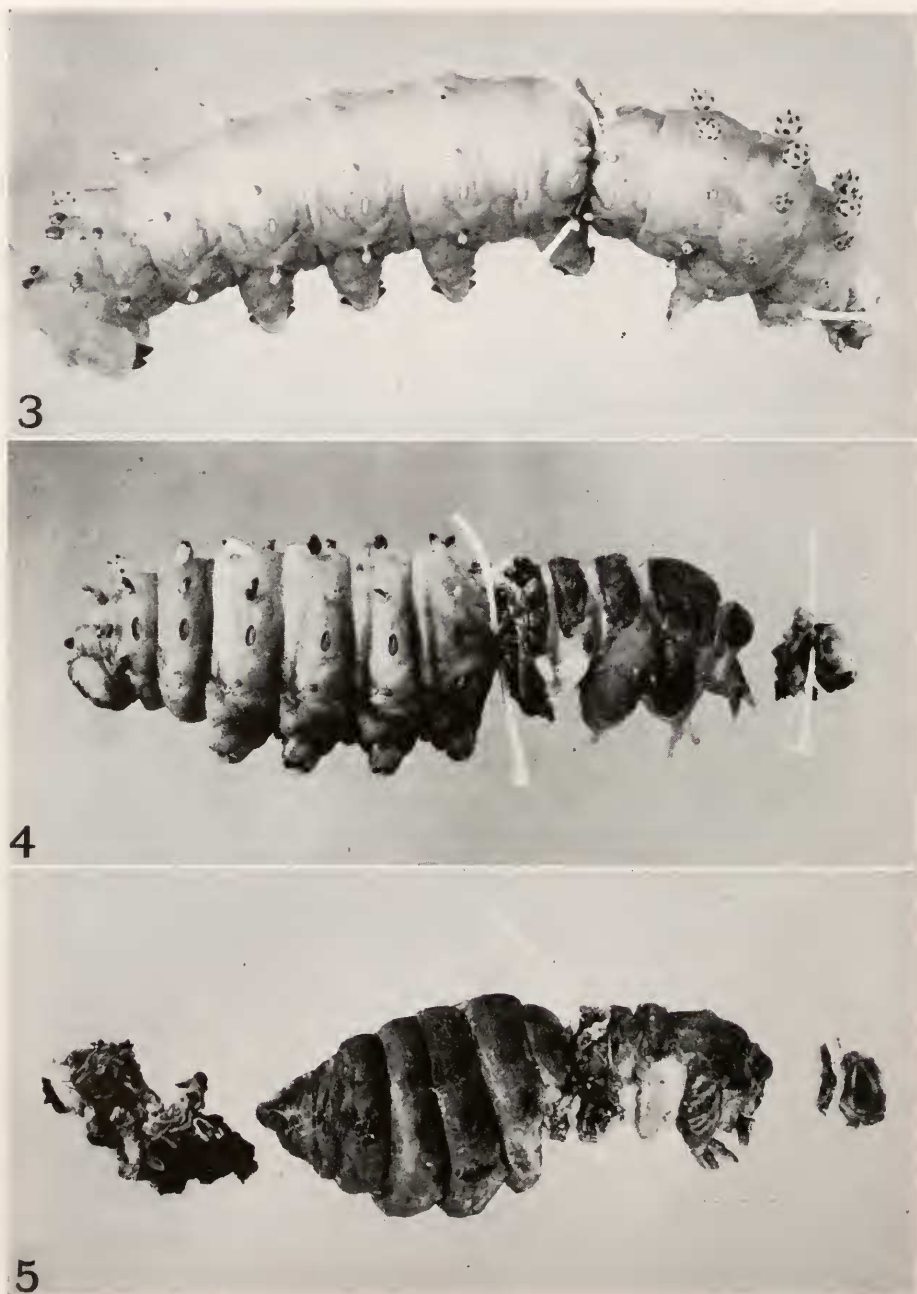
FIGURE 3. A mature Cecropia larva ligatured behind head and thorax prior to the initiation of spinning. Neither end is able to metamorphose.

FIGURE 4. The same as Figure 3, except that the ligatures were applied during the second day of spinning. The thorax has pupated, but the abdomen remains larval.

FIGURE 5. The same as Figure 3, except that the ligatures were applied after the initiation of the prepupal stage. Both the thorax and abdomen have pupated. The dead larval head and moulted larval cuticle are also illustrated.

in 17 of the 21 preparations. However, in animals ligated a day later, pupation of the abdominal compartment was consistently observed (Fig. 5).

These simple experiments demonstrate that pupation is under the control of both the head and the thorax. The head makes its contribution prior to a critical period signalled by the completion of the outer capsule of the cocoon and, thus, 9 days before the pupal moult. Pupation then comes under the control of the thorax until a critical period signalled by the onset of the prepupal stage.

TABLE II

Behavior of thoracic and abdominal compartments of double-ligatured Cecropia silkworms

Days at 25° C.	Corresponding stage	Number of experiments	Final state of	
			Thorax	Abdomen
0	Just prior to spinning	5	Larval	Larval
0 to 1*	Spinning outer capsule	14	Larval or pupal	Larval
1 to 2	Spinning inner capsule	18	Pupal	Larval
2	Just finished spinning	17	Pupal	Larval
4 to 5**	Earliest retraction of ocellar pigment	21	Pupal	Larval or pupal
5	Full retraction of ocellar pigment	4	Pupal	Pupal
5 to 9	Prepupa	4	Pupal	Pupal
9 to 10	Pupal moult	—	Pupal	Pupal

* "Critical period" for brain.
** "Critical period" for prothoracic glands.

Those thoracic or abdominal compartments which pupated underwent no further development though they continued to live for up to three months thereafter. This behavior is understandable since, as we have seen, the pupal-adult transformation once again requires the hormonal participation of a cephalic organ, the brain, and a thoracic organ, the prothoracic glands.

Ability of Larval Brains to Terminate Pupal Diapause

Efforts were made to determine whether the larval brain controls pupation *via* the same brain hormone, which, at a later stage in the life history, controls adult development. To this end, brains were dissected from caterpillars at precise stages, freed from frontal ganglia and other attached organs, and implanted into the tips of the abdomens of brainless diapausing pupae. Two brains were implanted into each preparation.

The results recorded in Table III demonstrate the ability of the larval brain to substitute for the pupal brain in terminating diapause. In one or more experiments, the brains were found active at every stage in larval and prepupal life.

However, it will also be observed in Table III that many of the brain implants failed to promote adult development and, even when they did so, considerable variation was encountered in the time required for the initiation of development.

When observed through an overlying plastic window, it became apparent that the implanted brains attained intimate connections with the tissues of the host prior to exerting their effect. Minute nerve fibers grew out from the brain and tracheal connections grew in from the surrounding tissues of the host. The subsequent history of such implants was therefore largely uncontrollable—a fact which was probably responsible for a large fraction of the observed variability.

TABLE III

Endocrine activity of larval brains implanted into brainless diapausing Cecropia pupae
(two brains into each)

Stage of donors	Number of experiments	Number developing	Days for initiation of development* at 25° C.
Mature 1st instar	2	1	50
1st moulting to 2d	3	1	195
Early 2d	2	2	20; 251
Mature 2d	3	1	17
2d moulting to 3d	2	2	13; 226
Early 3d	1	1	228
3d	2	2	10; 181
Mature 3d	3	2	21; 139
3d moulting to 4th	2	2	25; 217
Early 4th	2	2	28; 110
4th	2	2	48; 257
Mature 4th	4	3	16; 51; 173
4th moulting to 5th	4	3	65; 177
Early 5th	17	12	12; 18; 21; 23; 25; 47; 48; 58; 73; 112; 225;—
Mature 5th	18	9	11; 48; 107; 137; 143; 180; 218; 221; —
Spinning outer capsule	19	8	12; 17; 18; 28; 39; 55; 112; 190
Spinning inner capsule	13	6	65; 71; 200; 210; 240; 520
Finished spinning	11	5	65; 129; 134; 152; 166
Early retraction of ocellar pigment	6	5	80; 97; 130; 153; 187
Prepupa	7	5	64; 140; 159; 165; 225
Fresh pupa	10	0	—

* Positive experiments only.

Consequently, on the basis of the present data, it is impossible to judge whether any systematic change occurs in the brain's endocrine activity between the first larval stage and the initiation of spinning. However, it is clear that during the first day of spinning the brain is as active as at any stage in larval life. Yet, within the nine days that follow, a rapid decline takes place in its endocrine activity. By the time of the pupal moult, the brain is totally inactive when tested. The net result is that the newly formed pupa is equipped with a brain which is incompetent to secrete the brain hormone.

ABILITY OF LARVAL PROTHORACIC GLANDS TO TERMINATE PUPAL DIAPAUSE

According to the preceding analysis, the larval brain secretes the same brain hormone in promoting pupation as does the pupal brain in promoting adult de-

velopment. An extension of this principle to the prothoracic glands suggests that
the same prothoracic gland hormone controls both pupation and adult development.
This hypothesis was tested in brainless diapausing pupae by implanting prothoracic
glands obtained from caterpillars at various stages during late larval and prepupal
life. Two pairs of glands were implanted into each pupa.

As recorded in Table IV, larval glands removed prior to the initiation of spin-
ning caused adult development to occur in only two of the 23 preparations, whereas
glands removed after the initiation of spinning and prior to the prepupal period were
active in 7 of 11 preparations. Glands removed after the onset of the prepupal
stage were inactive.

TABLE IV

Endocrine activity of larval prothoracic glands implanted into brainless diapausing cecropia pupae
(two pairs into each)

Stage of donors	Number of experiments	Number developing	Days for initiation of development* at 25° C.
Mature 4th instar	4	0	—
4th moulting to 5th	2	0	—
Early 5th	9	1	27
Mature 5th	8	1	19
Spinning outer capsule	2	2	16; 21
Spinning inner capsule	5	2	15; 16
Finished spinning	3	3	23; 240; 480
Early retraction of ocellar pig-ment	3	0	—
Prepupa	1	0	—
Pupa	5	0	—

* Positive experiments only.

From these experiments we learn that the larval prothoracic glands can be made
to substitute for the pupal glands in terminating pupal diapause. Since the glands
were tested in brainless pupae, the experiments also demonstrate that the larval
glands usually exist in the activated state during a five-day period beginning with
the initiation of spinning and ending with the onset of the prepupal stage.

PUPATION INDUCED BY PUPAL ENDOCRINE ORGANS

In the experiments just considered, the larval brain and prothoracic glands
were able to provide the necessary hormonal stimulus for the adult development of
the pupa. Can the corresponding pupal organs evoke the pupation of the larva?

A preliminary series of experiments indicates an affirmative answer to this
question. In three of a total of six preparations, "permanent" larval abdomens,
isolated by ligation prior to spinning, were induced to pupate by implanting brains
and prothoracic glands obtained from chilled pupae.

Parabiosis Experiments

In our progress up to this point, support has been found for the view that the prothoracic glands secrete the metamorphosis hormone under tropic stimulation of the brain hormone. Further insight into the function of this endocrine system is afforded by experiments where brainless pupae were joined in serial parabiosis.

A series of Cecropia pupae were first stabilized in "permanent" diapause by removing their brains and replacing the pupal cuticle and hypodermis at the site of the operation by a transparent plastic facial window (Williams, 1946b, 1947). In like manner, plastic windows were established in each pupa at the thoracic tergum and the tip of the abdomen. A week later, a window was removed from each individual and the pupae united in pairs, the thorax of the one being sealed with melted paraffin to the tip of the abdomen of the other. Ten days later the pairs of pupae were united by a continuation of the above-mentioned procedure. In this manner, chains of four to ten brainless pupae were established in serial parabiosis.[3] In approximately a week the operative sites underwent a process of repair accompanied by a growing together of the hypodermis of successive individuals, yielding, as it were, a single elongate organism possessing continuity of blood and hypodermis. Though each individual retained prothoracic glands within its thorax, none possessed a brain within its head. For this reason the diapause was persistent and three chains of pupae died several months later without any indication of adult development.

Experiments were performed to ascertain the effects of implanting a brain into such preparations. Accordingly, a single brain from a previously chilled pupa was implanted under the facial window of the first animal in each chain, its effects being noted by daily observations through the transparent facial windows. The five viable preparations of this type yielded essentially similar results. The behavior of the preparation illustrated in Figures 6 and 7 may be summarized as follows:

0 day—brain implanted into ✳1.

17th day—✳1 and ✳2 show initiation of development; no development of ✳3 to ✳8.

20th day—✳3 and ✳4 show initiation of development; no development of ✳5 to ✳8.

21st day—✳5 shows initiation of development; no development of ✳6 to ✳8.

30th day—✳6 and ✳7 show initiation of development; no development of ✳8.

32nd day—✳8 shows initiation of development.

38th day—✳1 and ✳2 have completed adult development.

41st day—✳3, ✳4, and ✳5 have completed adult development.

50th day—✳6 and ✳7 have completed adult development.

52nd day—✳8 has completed adult development.

Thus we observe that the single brain set in motion a process of activation which terminated the diapause of each animal in turn. This process began at the anterior

[3] The principal difficulty in establishing these chains is that an infection or darkening of the blood in any one animal promptly spreads throughout the chain and causes the death of all individuals. It is therefore advisable to subdivide the total procedure into several stages spaced at least a week apart. In this manner one may confirm the viability of each animal before proceeding to the next step in assembling the chain.
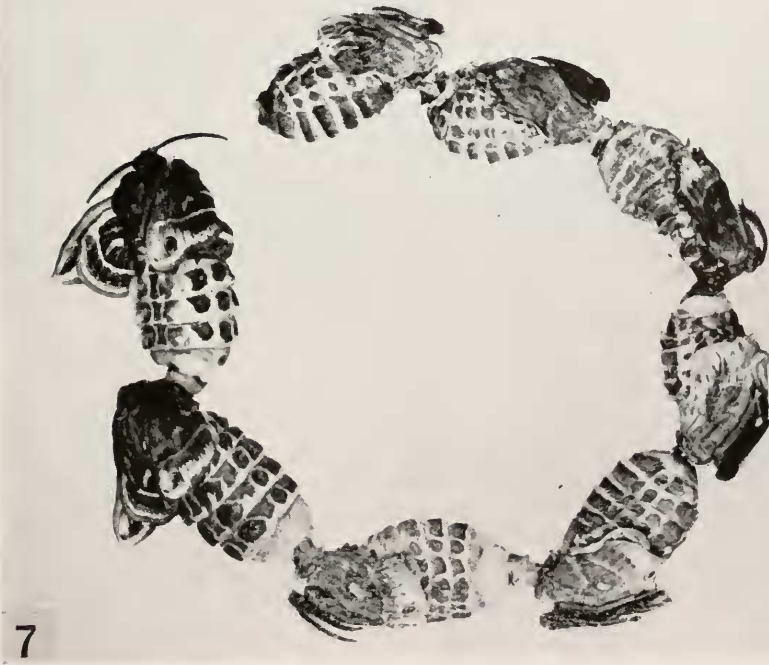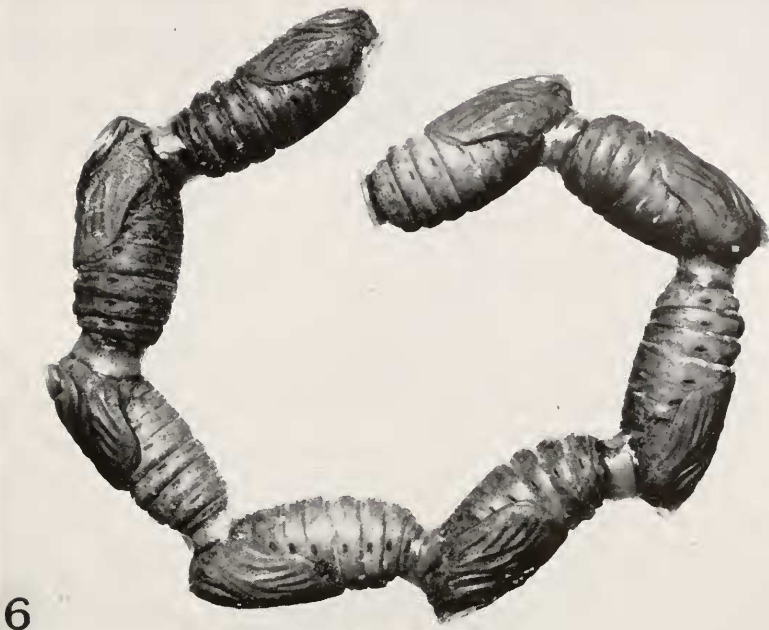
FIGURE 6. A chain of eight brainless diapausing pupae has been established and a single chilled pupal brain implanted under the facial window of the anterior-most individual. Approximately life size.

FIGURE 7. The preparation in Figure 6 after seven and one-half weeks. The single brain has caused all the pupae to undergo adult development.

end of the chain and required 15 days to travel 24 cm. to the tip of the eighth individual. The chain of pupae yielded a chain of moths, complete both externally and internally. Successive individuals were inter-connected at the site of parabiosis by a hollow bridge of integument.

Light is shed on the role of the brain in such preparations by the experiment illustrated in Figures 8 and 9. Here a chain of 6 brainless diapausing pupae was established and a brain implanted into ✻1. When ✻1 and ✻2 showed the earliest initiation of development, ✻1 containing the brain, and ✻6 were detached. As illustrated in Figure 9, the activation, now in the absence of the brain, continued beyond ✻2 and traversed the residual three pupae in the chain. All the animals completed adult development save ✻6; the latter died 18 months later without any indication of development.

The failure of the hindmost animal to develop after detachment indicates that a threshold titer of the brain hormone had not been distributed throughout the chain prior to the initiation of development of animals ✻1 and ✻2. Yet the detachment of the brain-containing pupa did not interfere with the further nondecremental spread of activation from animal ✻2 to ✻3 and from ✻3 to ✻4 and from ✻4 to ✻5. It seems necessary to conclude that the brain hormone acted locally at the anterior end of the chain to initiate a reaction which, without further dependency on the brain or the brain hormone, could be duplicated in each successive pupa.

The fact that each pupa contained prothoracic glands appeared to afford a rational basis for such a self-sustaining reaction. According to this interpretation, the brain hormone triggered the prothoracic glands of animal ✻1 and the latter's hormone then spread to animal ✻2 and triggered its prothoracic glands. A continuation of this process would lead to the recruitment of prothoracic gland activity in each successive pupa—the glands being triggered by the prothoracic gland hormone itself arising in the preceding pupa.

· Under this point of view, the self-sustaining character of the process should not appear if prothoracic glands were present only in the anterior-most insect. This prospect was tested experimentally.

Since it is technically impossible to extirpate the prothoracic glands of Cecropia, the experiment was performed on chains of isolated abdomens. Twenty chilled pupae were transected just behind the prothoracic glands; *i.e.*, at the meso-metathoracic level. Crystals of penicillin and streptomycin were implanted at this time to minimize the chance of infection. Each abdomen was then sealed with melted paraffin to a plastic slip containing a central hole 5 mm. in diameter; the latter was temporarily plugged with melted paraffin. Each abdomen was further provided with a plastic window at the tip of the abdomen. Twelve of the 20 abdomens survived this treatment and were in good condition a week later. The paraffin plugs and the abdominal windows were then removed and pairs of pupae

FIGURE 8. A chain of six brainless diapausing pupae has been established and a single chilled pupal brain implanted under the facial window of the anterior-most individual. When pupae 1 and 2 showed the initiation of development, pupae 1 and 6 were detached. Approximately life size.

FIGURE 9. The preparation in Figure 8 seven weeks later. After detachment of the brain-containing pupa, the activation has continued to spread down the chain of interconnected pupae. The hindmost pupa fails to develop after detachment. Approximately life size.
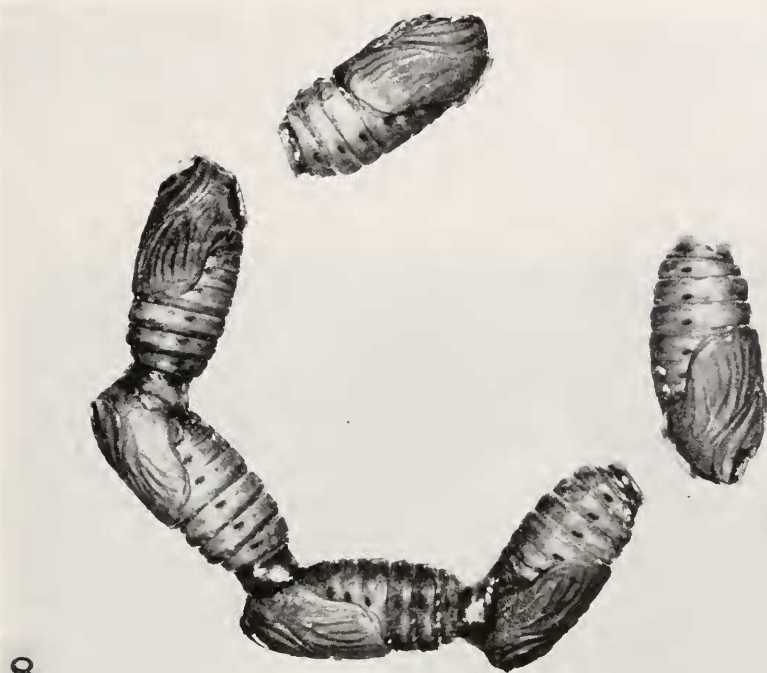
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established and sealed together with melted paraffin. Ten days later, the pairs were joined in sequence by a continuation of this procedure. Finally a normal chilled pupa was grafted to the anterior end of each chain of four abdomens (Fig. 10). Two such chains were prepared, plus one control preparation in which a chilled pupa was joined to a single abdomen.

In the case of the control preparation, the pupa initiated adult development on the 12th day and the attached abdomen on the 14th day. Both showed complete adult development on the 35th day.

FIGURE 10. A chilled pupa, containing both a brain and prothoracic glands, has been attached to a chain of four pupal abdomens lacking these endocrine organs. The activation here spreads decrementally: the anterior-most animals undergo adult development, the hindmost animals do not. Approximately life size.

One of the two experimental preparations survived. The pupa initiated development on the 14th day, abdomen ✳1 on the 15th day, and abdomen ✳2 on the 18th day. Abdomens ✳3 and ✳4 showed no development.

On the 38th day the pupa and abdomen ✳1 showed complete adult development. Abdomen ✳2 showed only the earliest stage of development, while abdomens ✳3 and ✳4 showed no development. The connections between successive abdomens remained patent and, by pressing on the abdomens, the blood could be propelled between the various members of the chain. The moth and the adult abdomen ✳1 were at this time detached from the chain. Though the latter survived until the 80th day, none of its members underwent further development.

The behavior of the chain of abdomens therefore stands in marked contrast to that observed in the previous series of experiments where each member of the chain possessed prothoracic glands. When each member possessed prothoracic glands,

then the activation spread without decrement and could apparently cause the development of any number of brainless pupae that one incorporated into the chain. But when only the anterior-most animal possessed prothoracic glands, then the activation spread decrementally and only the next adjacent member of the chain received the necessary concentration of prothoracic gland hormone.

Just such a difference in the behavior of the two types of preparations would be anticipated if the prothoracic glands of successive individuals can be triggered by the prothoracic gland hormone itself, arising in the preceding member of the chain. Manifestly, within the normal insect the operation of this mechanism would assist the integration of the endocrine mechanism. Since the prothoracic glands are paired organs, whose respective thresholds to brain hormone may differ, the sensitivity of one prothoracic gland to the other's hormone would serve to synchronize their secretory activities and couple the two glands into a functional unity.
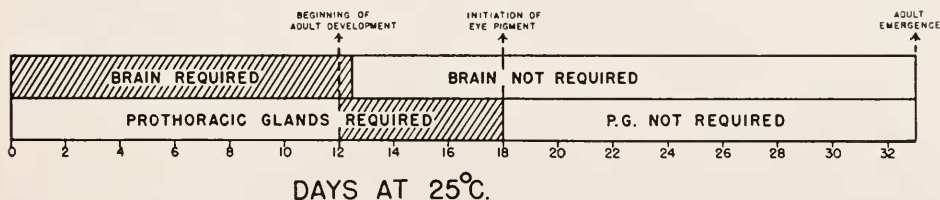
Discussion

The experimental results summarized in Figure 11 demonstrate that both pupation and adult development are controlled by an endocrine system consisting of the brain and prothoracic glands. According to the ligation experiments

A. PUPATION
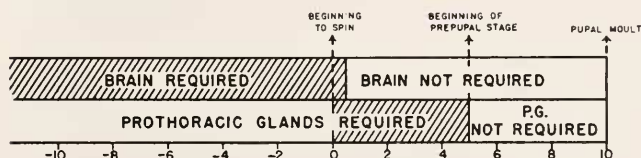
B. ADULT DEVELOPMENT

DAYS AT 25°C.

FIGURE 11. The endocrine control of the pupation of the larva (A) and the adult development of the previously chilled pupa (B) at 25° C. For explanation see text. The cross-hatching records the periods when the brain or prothoracic glands were found maximally active.

(Table II), pupation requires an initial stimulus from the larval brain and a subsequent stimulus from the larval prothoracic glands. As diagrammed in Figure 11A, the brain completes its contribution to pupation during the first day of spinning, i.e., 9 days prior to the pupal moult, and then declines in endocrine activity (Table III).

As also illustrated in Figure 11A, the termination of brain function ushers in a five-day period of prothoracic gland function, beginning with the onset of spinning and ending with the onset of the prepupal stage. The further development of the pupa then becomes independent of the prothoracic glands. The latter remain intact, but show a rapid loss of endocrine activity (Table IV).

Within the pupa the function of the prothoracic glands in promoting adult development once again requires the tropic stimulus of the brain hormone. But in the Cecropia silkworm, as we have seen, this stimulus is not forthcoming for several months after pupation, until the diapausing brain has recovered its endocrine competency under the influence of low environmental temperature.

When such a chilled pupa is placed at 25° C., the events which terminate its diapause are strikingly similar to those which controlled its pupation. As diagrammed in Figure 11B, the secretion of the brain hormone occurs promptly at the high temperature, proceeding at a rate determined largely by the duration of the brain's prior exposure to low temperature (Williams, 1952). The brain hormone attains threshold titer after a specific period, diagrammed as 12 days in Figure 11B. Then, within a few hours, a remarkable series of events takes place: (1) the prothoracic glands, which have been inactive since the prepupal stage, are triggered by the brain hormone; (2) the prothoracic gland hormone reacts with the pupal tissues to evoke the initiation of adult development (Table I); and (3) the brain becomes dispensable and undergoes a rapid decline in endocrine activity (Williams, 1947).

For six days following the termination of diapause, the further progress of adult development continues to require the secretory activity of the prothoracic glands. But, unlike their behavior prior to pupation, the glands undergo complete degeneration during this first week of adult development. Notwithstanding this fact, the prothoracic gland hormone apparently persists within the animal until adult emergence and is thus in a position to influence the later stages of adult development (Schmidt and Williams, 1949).

According to the preceding analysis, the pupal diapause is terminated by the recurrent function of the same endocrine system that controls pupation itself. Moreover, the demonstrated ability of larval brains and prothoracic glands to substitute for the corresponding pupal organs indicates that the same brain hormone and prothoracic gland hormone control both pupation and adult development.

In relation to both events we observe that the termination of brain function is synchronized with the initiation of a prothoracic gland function—a sequence which argues that the brain hormone is solely a tropic stimulus for the prothoracic glands. This inference is greatly strengthened by the finding that larval or pupal prothoracic glands, previously exposed to the brain hormone, were able to evoke adult development in the absence of the brain (Table I). Though the brain gives leadership to the endocrine events which preside over pupation and adult development, it apparently does so *via* the tropic action of its hormone on the prothoracic glands.

In agreement with Fukuda's investigation of *Bombyx mori* (1944), the present findings therefore serve to emphasize the significance of the prothoracic gland hormone, since it is apparently this factor which reacts with the larval tissues to induce pupation and with the pupal tissues to induce adult development.

Attempts to determine whether the brain and prothoracic glands also partici-
pate in the hormonal control of larval growth and moulting are complicated by
technical difficulties.   For as Bounhiol (1938) has emphasized, the moulting or
pupation of most insects becomes possible only when the animal attains a certain
nutritional status.   Each larval instar therefore begins with a period of *alimen-
tation indispensable,* followed by a period of *alimentation facultative.*   During
the first of these periods metamorphosis is blocked by any treatment which pre-
vents further feeding—such measures, for example, as ligation, brain removal,
or simple starvation.   In consequence, a direct approach to the analysis of the
hormonal control of moulting has proved feasible only in *Rhodnius* where, at the
beginning of each instar, the period of *alimentation indispensable* is limited to
a single blood meal (Wigglesworth, 1940, 1951).   Studies of this type have proved
difficult or impossible in most other insects where the hormonal control of moulting
is usually exercised during the period of *alimentation indispensable.*

Indeed, our understanding of the hormonal control of pupation is obscured by
this difficulty in certain species.   Unfortunately, this appears to be so in a favorite
experimental animal, *Bombyx mori.*   Here the brain apparently secretes a threshold
titer of its hormone early in the final larval instar when most experimental pro-
cedures are impossible.   This fact was adequately appreciated by Bounhiol (1938)
and probably accounts for Fukuda's (1944) failure to demonstrate a role of the
brain in the pupation of *B. mori.*

Notwithstanding these complications, there is circumstantial evidence that the
brain and prothoracic glands also participate in the regulation of larval growth
and moulting when, in the immature insect, they function in conjunction with the
corpora allata's "juvenile" or "status quo" hormone.   This conclusion is supported
by two lines of evidence: (1) the demonstrated ability of Cecropia brains to secrete
the brain hormone as early as the first larval instar; and (2) Fukuda's studies
(1944) of *B. mori* where the moulting of the immature insect appeared to require
the function of a prothoracic center.

Thus, at all stages in post-embryonic development, the picture which gradually
takes shape finds the prothoracic glands supplying an apparently direct stimulus
for cellular growth and differentiation.   Functioning in the presence of the corpora
allata's "juvenile" or "status quo" hormone, the prothoracic gland hormone pro-
motes the growth and moulting of the immature larva; functioning in the absence
of this conservative factor, it promotes the rapid strides in growth and differentia-
tion which culminate in the pupation of the mature larva and the adult development
of the pupa.

This conclusion based on studies of the Cecropia silkworm and its relatives is
in substantial agreement with Fukuda's (1944) results on *B. mori* and will prob-
ably apply to all Lepidoptera.   Indeed, recent studies of the Diptera (Possompes,
1949, 1950a, 1950b), the Blattoidea (Bodenstein, 1951), and Hemiptera (Wiggles-
worth, 1951) suggest that, in all metamorphosing insects, the prothoracic glands
and their homologues are the source of the factor which has variously been termed
the "moulting," "pupation," or "growth and differentiation" hormone.

Consequently, the control which the prothoracic gland hormone exercises over
pupation and adult development in the Cecropia silkworm is, most probably, the
expression of a generalized phenomenon.   The pupal diapause, under this point of
view, finds an endocrine basis in the latent operation of a normal mechanism shared

with non-diapausing species—the secretion of the prothoracic gland hormone. And according to the results of the present investigation, this latency, in turn, results from a loss of the endocrine activity of both the brain and the prothoracic glands during the prepupal period. Adult development becomes possible only when the brain recovers its secretory powers and repeats the tropic stimulation which it had administered to the prothoracic glands prior to the prepupal period.

In the case of non-diapausing species, where the prothoracic glands promote prompt adult development by secreting their hormone a few days following pupation, the timing of the endocrine events appears to be modified in one of three directions: (1) both the brain and prothoracic glands may remain active following pupation; or (2) the brain may remain active within the newly found pupa while the prothoracic glands become temporarily inactive; or (3) the brain may become inactive following pupation while the prothoracic glands remain active. All of these circumstances are consistent with the function or activation of the prothoracic glands and, therefore, with the prompt onset of adult development; all will probably be encountered when a sufficiently large number of non-diapausing species is examined.

In the case of species incapable of pupal diapause, the first or third adaptations appear to be the rule. Thus the adult development of *Bombyx* (Bounhiol, 1938; Fukuda, 1944), *Deilephila* (Caspari and Plagge, 1935; Plagge, 1938), *Galleria* (Bounhiol, 1938), *Lymantria* (Kopeć, 1922; Bounhiol, 1938), *Phryganidia* (Bodenstein, 1938), and *Vanessa* (Hachlow, 1931) is known to proceed normally in brainless pupae. Our studies show this to be true also in the non-diapausing pupae of the monarch butterfly, *Danaus plexippus*, and the southern armyworm, *Prodenia eridania*.

In the case of the bivoltine silkworm, *Actias luna*, and the polyvoltine *Actias selene*, the initial brood of non-diapausing pupae undergoes prompt adult development *via* the second adaptation mentioned above. Within the newly formed pupa the brain is active and the prothoracic glands inactive. The pupa is therefore converted to diapause if the brain is removed immediately after pupation. However, the subsequent diapausing brood of *Actias luna* shows the same timing of events as observed in Cecropia in that the brain as well as the prothoracic glands are inactive within the newly formed pupa.

Thus, in our understanding of the endocrinology of metamorphosis, we simultaneously perceive a simplification and an increasing complexity—simplification in the operation of a potent growth factor, the prothoracic gland hormone, in controlling growth and differentiation at all stages in post-embryonic development; increasing complexity in that secretion of this hormone is under the tropic control of the brain.

SUMMARY

1. The pupation and adult development of the Cecropia silkworm are under the control of a hormonal system consisting of the brain and prothoracic glands. The adult development of the pupa is controlled by the same "brain hormone" and "prothoracic gland hormone" which, at an earlier stage in the life history, control the pupation of the larva.

2. Though the brain gives leadership to the endocrine events that preside over pupation and adult development, it apparently does so by supplying a tropic hormonal stimulus for the prothoracic glands. The latter's "growth and differentiation hormone," secreted under stimulation of the brain hormone, then reacts with the larval tissues to promote pupation or with the pupal tissues to promote adult development.

3. The pupal diapause is a state of endocrine deficiency resulting from a temporary failure of the brain in secreting its tropic hormone following pupation. What the pupal tissues require is the prothoracic glands' growth and differentiation hormone. But this factor becomes available only after the brain recovers its secretory ability and triggers the function of the prothoracic glands.

4. Evidence is presented that the prothoracic glands can be triggered also by the prothoracic gland hormone itself. This device, it is suggested, serves to couple the paired glands into a functional unity and assure their simultaneous response when the brain hormone is released.

5. Evidence of a more circumstantial character indicates that larval growth and moulting are also promoted by the prothoracic glands' growth and differentiation hormone, acting within the immature insect in conjunction with a conservative factor secreted by the corpora allata. The corpus allatum hormone plays no positive role in the pupation or adult development of the Cecropia silkworm.

6. The endocrine system of Cecropia is compared to that of bivoltine and non-diapausing Lepidoptera. In Cecropia, both the brain and prothoracic glands become inactive after providing the endocrine stimulus for pupation. Prompt, non-diapausing development becomes possible in those species where either the brain or the prothoracic glands retain their endocrine activity within the newly formed pupa.

7. The pupal diapause therefore results from the delayed function of a normal endocrine mechanism shared with non-diapausing species.

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